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ORIGINAL ARTICLE

Anemia and hematinic deficiencies in anti-gastric parietal cell antibody-positive or all autoantibodies-negative recurrent aphthous stomatitis patients

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Vitamin B12 deficiency

Background/purpose: Approximately 13% of recurrent aphthous stomatitis (RAS) patients have serum anti-gastric parietal cell antibody (GPCA) positivity. This study assessed whether serum GPCA or RAS itself was a significant factor causing hematinic deficiencies and anemia statuses in GPCA-positive RAS (GPCA+/RAS) and all autoantibodies-negative RAS (Abs−/RAS) patients.
Methods: The mean corpuscular volume (MCV) and mean blood hemoglobin (Hb), iron, vitamin B12, and folic acid levels were measured and compared between any two of three groups of 31 GPCA+/RAS patients, 240 Abs−/RAS patients, and 342 healthy control subjects.
Results: GPCA+/RAS patients had significantly lower mean Hb and serum iron level (for women only) as well as significantly greater frequencies of Hb, iron, and vitamin B12 deficiencies than healthy control subjects. Moreover, GPCA+/RAS patients had a significantly higher MCV and a significantly greater frequency of vitamin B12 deficiency than Abs−/RAS patients. Furthermore, Abs−/RAS patients did have significantly lower mean Hb, MCV, iron, and folic acid levels and significantly greater frequencies of Hb, iron, vitamin B12, and folic acid deficiencies than

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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healthy control subjects. Of 31 GPCA+/RAS patients, 3 (9.7%) had PA, 6 (19.4%) had vitamin B12 deficiency, and 3 (9.7%) had macrocytosis. Moreover, normocytic anemia (54.0%) and iron deficiency anemia (26.4%) are the two more common types of anemia in our RAS patients.
Conclusions: We conclude that serum GPCA plays a significant role in causing vitamin B12 deficiency and high MCV in GPCA+/RAS patients. RAS itself does play a significant role in causing anemia and hematinic deficiencies in both GPCA+/RAS and Abs−/RAS patients.

Introduction

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disease characterized by recurrent and painful ulcerations on the nonkeratinized oral mucosae such as labial, buccal, alveolar, and ventral tongue mucosae. In Taiwan, the prevalence of RAS is 10.5% in the general population.[1]

Although several etiological factors have been proposed, the exact causes of RAS are still not very clear.[2] Previous studies of tissue infiltrated mononuclear cells in RAS specimens favor the role of cell-mediated cytotoxicity in the immunopathogenesis of RAS.[3] In addition to immune dysregulation, multiple nutritional deficiencies including deficiencies of vitamins B1, B2, B6, and B12, folate, iron, and ferritin are reported to be the possible etiologies of RAS.[3] Our previous studies showed that 57 (20.9%), 55 (20.1%), 13 (4.8%) and 7 (2.6%) of 273 RAS patients have deficiencies of hemoglobin (Hb), iron, vitamin B12, and folic acid, respectively.[3] We also demonstrated that 13.0%, 19.4%, and 19.7% of 355 RAS patients had the presence of anti-gastric parietal cell (GPCA), anti-thyroglobulin (TGA), and anti-thyroid microsomal autoantibodies (TMA) in their sera, respectively.[4] It is well known that GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor production[5,6] and vitamin B12 deficiency that finally leads to the status of pernicious anemia (PA).[7,8] Vitamin B12 deficiency may also be due to insufficient intake of vitamin B12-containing foods, vitamin B12 malabsorption, and transcobalamin II deficiency.[8] Because multiple factors are involved in Hb deficiency (anemia) in RAS patients, it is interesting to know what factors are most important for the development of anemia or hematinic deficiencies in GPCA-positive RAS (GPCA+/RAS) patients and all autoantibodies-negative RAS (Abs−/RAS) patients.

In our oral mucosal disease clinic, patients with atrophic glossitis (AG), burning mouth syndrome (BMS), oral lichen planus (OLP), RAS, oral submucous fibrosis (OSF), and other oral mucosal diseases are frequently encountered.[3,4,9–28] For AG, BMS, OLP, RAS and OSF patients, complete blood count, serum iron, vitamin B12, folic acid, homocysteine, serum anti-nuclear autoantibody (ANA), anti-smooth muscle autoantibody (SMA), anti-mitochondrial autoantibody (AMA), GPCA, TGA, and TMA levels were frequently examined to assess whether these patients have anemia, hematinic deficiencies, and serum ANA, SMA, AMA, GPCA, TGA, or TMA positivity.[3,4,9–25] To assess the roles of GPCA positivity and the disease of RAS itself in the development of anemia and hematinic deficiencies in GPCA+/RAS and Abs−/RAS patients, 31 GPCA+/RAS patients without ANA, SMA, AMA, TGA and TMA positivities and 240 Ab-/RAS patients were collected. Their complete blood counts as well as serum iron, vitamin B12, folic acid, and homocysteine levels were examined and compared with the corresponding data of 342 healthy control subjects without ANA, SMA, AMA, GPCA, TGA and TMA positivities. The purposes of this study were to evaluate the hematinic deficiencies and anemia statuses in these 31 GPCA+/RAS patients and 240 Abs−/RAS patients and to clarify the roles of the serum GPCA and/or the disease of RAS itself in the final development of anemia and hematinic deficiencies in our GPCA+/RAS and Abs−/RAS patients.

Materials and methods

Subjects

In this study, 31 (10 men and 21 women, age range 24–90 years, mean age 63.3 ± 12.9 years) GPCA+/RAS patients and 240 (78 men and 162 women, age range 18–90 years, mean age 50.9 ± 16.3 years) Abs−/RAS patients were collected from the oral mucosal disease clinic of National Taiwan University Hospital (NTUH). For comparisons, 342 healthy control subjects (104 men and 238 women, age range 20–89 years, mean age 52.7 ± 14.7 years) were also collected and included in this study. All RAS patients and control subjects were seen consecutively, diagnosed, and treated in the Department of Dentistry, NTUH from July 2007 to July 2016. Patients were diagnosed as having RAS when they had at least one episode of oral ulcerations on movable oral mucosa per month since childhood.[3] RAS patients with betel quid chewing habit or autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid) were excluded. Moreover, patients with traumatic ulcers or with aphthous-like ulcers associated with systemic disorders including Behcet's syndrome, celiac disease, gluten-sensitive enteropathy, inflammatory bowel diseases, human immunodeficiency virus infection, and cyclic neutropenia were also excluded.[29] In addition, RAS patients with serum creatinine concentrations indicative of renal dysfunction (i.e., men, >131 μmol/L; women, >115 μmol/L), and who reported a history of stroke, heavy alcohol use, or diseases of the liver, kidney, or coronary arteries were also excluded.[30] Healthy

control subjects had either dental caries, pulpal disease, malocclusion, or missing of teeth but did not have any oral mucosal or systemic diseases. None of the RAS patients had taken any prescription medication for RAS at least 3 months before entering the study.

The blood samples were drawn from all included RAS patients and healthy control subjects for measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations as well as serum ANA, SMA, AMA, GPCA, TGA, and TMA levels. All RAS patients and healthy control subjects signed the informed consents before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH.

Determination of complete blood count and serum iron, vitamin B12, folic acid and homocysteine concentrations

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine concentrations were determined by the routine tests performed in the Department of Laboratory Medicine of NTUH as described previously.[3,10–25] This study defined the Hb and hematinic deficiencies according to the World Health Organization (WHO) criteria. Thus, men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL were defined as having Hb deficiency or anemia.[20,31] Patients with serum iron level $<$60 μg/dL,[32] vitamin B12 level $<$200 pg/mL[30] or folic acid level $<$4 ng/mL[33] were defined as having iron, vitamin B12 or folic acid deficiency, respectively. Moreover, patients with the serum homocysteine level $>$12.3 μM (which was the mean serum homocysteine level of healthy control subjects plus two standard deviations) were defined as having abnormally high homocysteine level.

Determination of serum ANA, SMA, AMA, GPCA, TGA, and TMA levels

The methods of determination of serum ANA, SMA, AMA, GPCA, TGA, and TMA levels in our RAS patients and healthy control subjects have been described in our previous studies.[4,9–14,17]

Statistical analysis

Comparisons of the mean MCV and mean blood levels of Hb, iron, vitamin B12, folic acid and homocysteine between GPCA+/RAS patients or Abs−/RAS patients and healthy control subjects and between GPCA+/RAS patients and Abs−/RAS patients were performed by Student's *t*-test. The differences in frequency of Hb, iron, vitamin B12 or folic acid deficiency, or frequency of abnormally high blood homocysteine level between GPCA+/RAS patients or Abs−/RAS patients and healthy control subjects and between GPCA+/RAS patients and Abs−/RAS patients were compared by chi-square test. Comparisons of mean MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between any two of the three groups of patients with macrocytic, normocytic or microcytic RBCs were performed by Student's *t*-test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The mean MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 31 GPCA+/RAS patients, 240 Abs−/RAS patients, and 342 healthy control subjects are shown in Table 1. Because men usually had higher blood levels of Hb and iron than women, these two mean levels were calculated separately for men and women. We found significantly lower mean Hb (for men and women, both *P*-values $<$ 0.001) and serum iron level (for women only; $P = 0.014$) as well as significantly higher mean blood homocysteine level ($P < 0.001$) in GPCA+/RAS patients than in healthy control subjects (Table 1). Moreover, we found a significantly higher mean MCV ($P = 0.002$) and folic acid level ($P = 0.039$) as well as a significantly higher mean blood homocysteine level ($P = 0.012$) in GPCA+/RAS patients than in Abs−/RAS patients. Furthermore, Abs−/RAS patients did have significantly lower mean Hb (for men and women, both *P*-values $<$ 0.001), MCV ($P < 0.001$), serum iron (for men and women, both *P*-values $<$ 0.001), vitamin B12 ($P = 0.072$, marginal significance), and folic acid ($P = 0.005$) levels than healthy control subjects (Table 1).

We also found significantly greater frequencies of Hb, serum iron and vitamin B12 deficiencies and of high blood homocysteine level in GPCA+/RAS patients than in healthy control subjects (all *P*-values $<$ 0.001) (Table 2). There were also significantly greater frequencies of vitamin B12 deficiency ($P = 0.037$) and of high serum homocysteine level ($P = 0.077$, marginal significance) in GPCA+/RAS patients than in Abs−/RAS patients. Moreover, we also demonstrated significantly greater frequencies of Hb, serum iron, vitamin B12, and folic acid deficiencies and of high blood homocysteine level (all *P*-values $<$ 0.001) in Abs−/RAS patients than in healthy control subjects (Table 2).

In this study, 10 (32.3%) of 31 GPCA+/RAS patients and 77 (32.1%) of 240 Abs−/RAS patients were diagnosed as having anemia according to the WHO criteria (Table 3).[31] The different anemia types of 10 anemic GPCA+/RAS patients and of 77 anemic Abs−/RAS patients are shown in detail in Table 3. In this study, PA was diagnosed as having MCV $\geq$ 100 fl, vitamin B12 $<$ 200 pg/mL, and serum GPCA positivity,[17–19] iron deficiency anemia as having MCV $<$ 80 fl and iron $<$60 μg/dL[31,32]; and thalassemia trait as having MCV $<$ 74 fl, RBC count $>5.0 \times 10^{12}$/L, and Mentzer index (MCV/RBC) $<$ 13.[23] By these definitions, of 10 anemic GPCA+/RAS patients, 3 had PA, 4 had normocytic anemia, 2 had iron deficiency anemia, and one had microcytic anemia but did not have iron deficiency and thalassemia trait. Moreover, of 77 anemic Abs−/RAS patients, 6 had macrocytic anemia, 43 had normocytic anemia, 21 had iron deficiency anemia, 5 had thalassemia trait, and two had microcytic anemia but did not have iron deficiency.

When 31 GPCA+/RAS patients were further divided into three groups: group 1 (3 patients with MCV $\geq$ 100 fl), group 2 (25 patients with MCV between 80 fl and 99.9 fl), and group 3 (3 patients with MCV $<$ 80 fl), we found that group 1

Table 1 Comparisons of the mean corpuscular volume (MCV) and mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid and homocysteine between any two of the three groups of 31 anti-gastric parietal cell antibody (GPCA)-positive recurrent aphthous stomatitis (RAS) patients (GPCA+/RAS patients), 240 all autoantibodies-negative RAS patients (Abs−/RAS patients), and 342 healthy control subjects.

Group	Hb (g/dL)		MCV (fl)	Iron (μg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
	Men	Women		Men	Women			
GPCA+/RAS patients (n = 31)	13.9 ± 1.9 (n = 10)	12.8 ± 1.0 (n = 21)	92.2 ± 9.5	102.7 ± 39.8 (n = 10)	82.9 ± 30.8 (n = 21)	613.7 ± 314.8	15.3 ± 6.1	12.8 ± 12.7
P-value[a]	<0.001	<0.001	0.074	0.858	0.014	0.174	0.280	<0.001
P-value[b]	0.730	0.343	0.002	0.252	0.854	0.637	0.039	0.012
Abs−/RAS patients (n = 240)	14.1 ± 1.7 (n = 78)	12.5 ± 1.4 (n = 162)	87.2 ± 8.1	88.8 ± 35.4 (n = 78)	81.4 ± 35.6 (n = 162)	638.4 ± 268.6	12.6 ± 6.9	8.7 ± 7.8
P-value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	0.072	0.005	0.649
Healthy control subjects (n = 342)	15.2 ± 0.7 (n = 104)	13.6 ± 0.7 (n = 238)	90.7 ± 3.7	104.4 ± 27.4 (n = 104)	98.8 ± 28.1 (n = 238)	676.5 ± 238.6	14.1 ± 5.9	8.5 ± 1.9

[a] Comparisons of parameters between 31 GPCA+/RAS patients or 240 Abs−/RAS patients and 342 healthy control subjects.
[b] Comparisons of parameters between 31 GPCA+/RAS patients and 240 Abs−/RAS patients.

Table 2 Comparisons of frequencies of hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies and frequency of abnormally high blood homocysteine level between any two of the three groups of 31 anti-gastric parietal cell antibody (GPCA)-positive recurrent aphthous stomatitis (RAS) patients (GPCA+/RAS patients), 240 all autoantibodies-negative RAS patients (Abs−/RAS patients), and 342 healthy control subjects.

	Hb deficiency (Men < 13 g/dL, Women < 12 g/dL)	Iron deficiency (<60 μg/dL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	High homocysteine level (>12.3 μM)
GPCA+/RAS patients (n = 31)	10 (32.3%)	6 (19.4%)	6 (19.4%)	0 (0.0%)	7 (22.6%)
P-value[a]	<0.001	<0.001	<0.001	ND	<0.001
P-value[b]	0.853	0.426	0.037	0.418	0.077
Abs−/RAS patients (n = 240)	77 (32.1%)	67 (27.9)	16 (6.7%)	12 (5%)	24 (10%)
P-value[a]	<0.001	<0.001	<0.001	<0.001	<0.001
Healthy control subjects (n = 342)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.9%)

ND = not done.
[a] Comparisons of parameters between 31 GPCA+/RAS patients or 240 Abs−/RAS patients and 342 healthy control subjects.
[b] Comparisons of parameters between 31 GPCA+/RAS patients and 240 Abs−/RAS patients.

patients had significantly higher MCV ($P < 0.001$), lower mean Hb (for men only, $P = 0.007$) and serum vitamin B12 ($P = 0.002$) levels, as well as higher mean serum folic acid ($P = 0.006$) and homocysteine ($P < 0.001$) levels than group 2 patients (Table 4). Moreover, group 3 patients had significantly lower mean Hb level (for women only, $P = 0.004$), lower MCV ($P < 0.001$), lower mean serum iron level (for women only, $P = 0.027$), and higher mean blood homocysteine level ($P = 0.002$) than group 2 patients (Table 4). In addition, group 1 patients had significantly higher MCV ($P < 0.001$) than group 3 patients (Table 4).

We also divided 240 Abs−/RAS patients into three groups: group 1 (6 patients with MCV ≥ 100 fl), group 2 (200 patients with MCV between 80 fl and 99.9 fl), and group 3 (34 patients with MCV < 80 fl). We found that group 1 patients had significantly higher MCV ($P < 0.001$), lower mean Hb (for men, $P < 0.001$; for women, $P = 0.049$) and serum vitamin B12 ($P < 0.001$) levels, and higher mean serum folic acid ($P = 0.050$, marginal significance) and homocysteine ($P < 0.001$) levels than group 2 patients (Table 5). Moreover, group 3 patients had significantly lower mean Hb level (for men and women, both *P*-values < 0.001), lower MCV ($P < 0.001$), lower mean serum iron level (for men, $P = 0.006$, for women, $P < 0.001$), and lower mean serum folic acid level ($P = 0.015$) than group 2 patients (Table 5). In addition, group 1 patients had significantly higher MCV ($P < 0.001$), higher mean serum iron level (for women only, $P = 0.033$), lower mean serum vitamin B12 ($P = 0.028$), and higher mean serum folic acid ($P = 0.004$) and homocysteine ($P < 0.001$) levels than group 3 patients (Table 5).

Discussion

This study found that GPCA+/RAS patients had significantly lower mean Hb and serum iron level (for women only), and

Table 3 Anemia types of 10 of 31 anti-gastric parietal cell antibody (GPCA)-positive recurrent aphthous stomatitis (RAS) patients (GPCA+/RAS patients) and of 77 of 240 all autoantibodies-negative RAS patients (Abs−/RAS patients).

Anemia type	Patient number (%)				
	Patient number (%)	MCV	Vitamin B12 deficiency (<200 pg/mL)	Iron deficiency (<60 μg/dL)	Folic acid deficiency (<4 ng/mL)
GPCA+/RAS patients (n = 31)					
Pernicious anemia	3 (30.0)	≥100 fl	3 (100.0)	0 (0.0)	0 (0.0)
Normocytic anemia	4 (40.0)	80–99.9 fl	0 (0.0)	2 (50.0)	0 (0.0)
Iron deficiency anemia	2 (20.0)	<80 fl	1 (50.0)	2 (100.0)	0 (0.0)
Microcytic anemia	1 (10.0)	<80 fl	1 (100.0)	0 (0.0)	0 (0.0)
Total	10 (100.0)		5 (50.0)	4 (40.0)	0 (0.0)
Abs−/RAS patients (n = 240)					
Macrocytic anemia	6 (7.8)	≥100 fl	5 (83.3)	1 (16.7)	0 (0.0)
Normocytic anemia	43 (55.8)	80–99.9 fl	3 (7.0)	23 (53.5)	3 (7.0)
Iron deficiency anemia	21 (27.3)	<80 fl	2 (9.5)	21 (100.0)	3 (14.3)
Thalassemia trait	5 (6.5)	<74 fl	0 (0.0)	0 (0.0)	0 (0.0)
Microcytic anemia	2 (2.6)	<80 fl	0 (0.0)	0 (0.0)	1 (50.0)
Total	77 (100.0)		10 (13.0)	45 (58.4)	7 (9.1)

Table 4 The mean corpuscular volume (MCV), mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine in 31 anti-gastric parietal cell antibody (GPCA)-positive recurrent aphthous stomatitis (RAS) patients (GPCA+/RAS patients), including 3 with MCV ≥ 100 fl (group 1), 25 with MCV between 80 fl and 99.9 fl (group 2), and 3 with MCV < 80 fl (group 3).

	GPCA+/RAS patients (n = 31)					
	Group 1 MCV ≥ 100 fl (n = 3)	*P*-value[a] (Group 1 vs. 2)	Group 2 MCV 80-99.9 fl (n = 25)	*P*-value[a] (Group 2 vs. 3)	Group 3 MCV < 80 fl (n = 3)	*P*-value[a] (Group 1 vs. 3)
	Mean ± SD		Mean ± SD		Mean ± SD	
Hb (g/dL)						
Men	11.3 ± 0.0 (n = 2)	0.007	14.9 ± 1.3 (n = 7)	ND	12.0 (n = 1)	ND
Women	11.3 (n = 1)	ND	13.1 ± 0.9 (n = 18)	0.004	10.8 ± 1.5 (n = 2)	ND
MCV (fl)	114.5 ± 5.3	<0.001	91.5 ± 3.4	<0.001	75.6 ± 2.8	<0.001
Iron (μg/dL)						
Men	77.5 ± 3.5 (n = 2)	0.430	104.9 ± 44.1 (n = 7)	ND	138.0 (n = 1)	ND
Women	91 (n = 1)	ND	87.5 ± 28.7 (n = 18)	0.027	37.0 ± 15.6 (n = 2)	ND
Vitamin B12 (pg/mL)	150.0 ± 0.0	0.002	689.6 ± 261.1	0.171	445.0 ± 481	0.348
Folic acid (ng/mL)	24.0 ± 0.0	0.006	14.0 ± 5.7	0.450	16.7 ± 6.4	0.119
Homocysteine (μM)	32.6 ± 24.2	<0.001	8.9 ± 3.1	0.002	25.3 ± 26.7	0.743

ND = not done.
[a] Comparisons of MCV, mean blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between groups 1 and 2, between groups 2 and 3, and between groups 1 and 3 by Student's *t*-test.

significantly higher mean serum homocysteine level than healthy control subjects. Moreover, GPCA+/RAS patients had significantly greater frequencies of Hb, serum iron, and serum vitamin B12 deficiencies and of high serum homocysteine level than healthy control subjects. These two findings suggest that the anemia status and hematinic deficiencies in GPCA+/RAS patients can be attributed to either serum GPCA positivity or the disease of RAS itself or both. To elucidate these equivocal probabilities, we compared the blood data between 31 GPCA+/RAS patients

Table 5 The mean corpuscular volume (MCV), mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine in 240 all autoantibodies-negative recurrent aphthous stomatitis (RAS) patients (Abs−/RAS patients) including 6 with MCV ≥ 100 fl (group 1), 200 with MCV between 80 fl and 99.9 fl (group 2), and 34 with MCV < 80 fl (group 3).

	Abs−/RAS patients (n = 240)					
	Group 1 MCV ≥ 100 fl (n = 6)	*P*-value[a] (Group 1 vs. 2)	Group 2 MCV 80-99.9 fl (n = 200)	*P*-value[a] (Group 2 vs. 3)	Group 3 MCV < 80 fl (n = 34)	*P*-value[a] (Group 1 vs. 3)
	Mean ± SD		Mean ± SD		Mean ± SD	
Hb (g/dL)						
Men	11.8 ± 0.9 (n = 4)	<0.001	14.5 ± 1.4 (n = 66)	<0.001	12.3 ± 1.6 (n = 8)	0.580
Women	11.1 ± 1.2 (n = 2)	0.049	12.8 ± 1.2 (n = 134)	<0.001	10.9 ± 1.1 (n = 26)	0.807
MCV (fl)	107.4 ± 4.9	<0.001	89.2 ± 3.9	<0.001	71.9 ± 6.1	<0.001
Iron (μg/dL)						
Men	110.0 ± 78.5 (n = 4)	0.296	91.2 ± 31.2 (n = 66)	0.006	58.5 ± 28.0 (n = 8)	0.117
Women	126.0 ± 72.1 (n = 2)	0.077	86.2 ± 30.8 (n = 134)	<0.001	53.5 ± 42.5 (n = 26)	0.033
Vitamin B12 (pg/mL)	294.8 ± 300.0	<0.001	658.3 ± 258.4	0.118	582.2 ± 281.0	0.028
Folic acid (ng/mL)	18.5 ± 5.1	0.050	12.9 ± 6.9	0.015	9.8 ± 6.5	0.004
Homocysteine (μM)	33.8 ± 39.7	<0.001	8.0 ± 3.4	0.429	8.5 ± 3.4	<0.001

[a] Comparisons of MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between groups 1 and 2, between groups 2 and 3, and between groups 1 and 3 by Student's *t*-test.

and 240 Abs−/RAS patients. We found GPCA+/RAS patients did have significantly higher MCV and higher serum homocysteine level as well as significantly greater frequencies of vitamin B12 deficiency and of high serum homocysteine level than Abs−/RAS patients. These findings indicate that the serum GPCA plays an important role in causing vitamin B12 deficiency, higher MCV, and higher homocysteine level in GPCA+/RAS patients. Although the serum GPCA has some contributions to cause the anemia, it does not play a significant role in causing anemia in GPCA+/RAS patients. Furthermore, this study also found that Abs−/RAS patients did have significantly lower mean Hb, MCV, serum iron, and folic acid levels as well as significantly greater frequencies of Hb, iron, vitamin B12, and folic acid deficiencies and of high blood homocysteine level than healthy control subjects. These findings indicate that the disease of RAS itself does play a significant role in causing anemia, hematinic deficiencies, and high blood homocysteine level in Abs−/RAS patients.

The serum GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor production[5,6] and vitamin B12 deficiency that finally leads to macrocytosis and the status of PA.[7,8] Our previous studies demonstrated that vitamin B12 and folic acid deficiencies can lead to high serum homocysteine level in oral mucosal disease patients.[3,10-18] Supplementation of multiple B vitamins especially vitamin B12 and folic acid can reduce the serum homocysteine levels in patients with AG or BMS.[24,34] Thus, the higher frequency of vitamin B12 deficiency, higher MCV, and higher homocysteine level in GPCA+/RAS patients than in healthy control patients are predominantly due to the presence of GPCA in GPCA+/RAS patients' sera.

Here, we explained why anemia and hematinic deficiencies may be etiological factors for the disease of RAS. Our previous study showed Hb, serum iron, vitamin B12, and folic acid deficiencies in 57 (20.9%), 55 (20.1%), 13 (4.8%), and 7 (2.6%) of 273 RAS patients, respectively.[3] In addition, a high homocysteine level was found in 21 (7.7%) of 273 RAS patients.[3] Our previous RAS study also described deficiencies of vitamins B1, B2, B6 and B12, folate, iron, ferritin and Hb in a portion of RAS patients.[3] Iron deficiency causes microcytic anemia[32] and deficiencies of vitamin B12 and folic acid lead to macrocytic anemia.[19,30] RAS patients with anemia and lower Hb levels have reduced capacity of the blood to carry oxygen to oral mucosa, finally resulting in atrophy of oral mucosa. In addition, iron is essential to the normal functioning of oral epithelial cells[35] and both vitamin B12 and folic acid play important roles in DNA synthesis and cell division.[3,30] Oral epithelial cells have a high turnover rate. Therefore, deficiencies of iron, vitamin B12, and folic acid may result in oral epithelial atrophy. Atrophic oral epithelium in hematinic-deficient patients may explain why some patients with deficiencies of hematinics are prone to have RAS. Furthermore, high blood homocysteine level may result in an elevated frequency of thrombosis in the feeding arterioles that supply the oral epithelial cells.[36–40] This in turn leads to a breakdown of oral epithelium and finally produces an oral ulceration. Moreover, replacement therapy with hematinics for RAS patients with deficiencies of corresponding hematinics can result in a significant clinical improvement or at least a reduction in frequency and severity of their oral aphthous ulcers.[41–44] Taken these together, anemia, hematinic deficiencies, and high blood homocysteine levels can decrease oral epithelial barrier and thus increase the frequency of RAS occurrence. Moreover, Hb and hematinic deficiencies and high blood homocysteine level may be the important etiological factors for RAS.

We further explain why RAS itself may be an important factor causing anemia and hematinic deficiencies in RAS patients. The results of a previous diet history questionnaire study indicate a significantly lower daily intake of vitamin B12 and folate in minor-typed RAS patients than in control subjects.[45] These results suggest that even the small oral ulcerations may impede the food intake by minor-typed RAS patients. By definition, the major-typed RAS patients often have severer and larger recurrent oral ulcerations than minor-typed RAS patients.[3] These severer ulcerative lesions may cause burning sensation and pain of the lesional oral mucosae when the patients eat salty and spicy food stuffs. The eating difficulty may result in reduced food intake that in turn leads to anemia and hematinic deficiencies in a certain percentage of our RAS patients.

In this study, 10 (32.3%) of the 31 GPCA+/RAS patients and 77 (32.1%) of 240 Ab-/RAS patients had anemia.[20,31] Six types of anemia including pernicious, macrocytic, normocytic, iron deficiency, and microcytic anemia and thalassemia trait were detected in RAS patients. We also found that 3 of 3 PA patients and 5 of 6 macrocytic anemia patients (one patient had concomitant iron deficiency) had vitamin B12 but not folic acid deficiency, suggesting that these two types of anemia are predominantly due to vitamin B12 deficiency. Of 47 normocytic anemia patients, 25 (53.2%) had iron deficiency, 3 (6.4%) had vitamin B12 deficiency, and 3 (6.4%) had folic acid deficiency, indicating that normocytic anemia in our RAS patients are predominantly due to iron deficiency and secondarily due to vitamin B12 and folic acid deficiencies. In our 23 iron deficiency anemia patients, 3 (13.0%) had concomitant vitamin B12 deficiency and another 3 (13.0%) had folic acid deficiency, suggesting that except for iron deficiency, both vitamin B12 and folic acid deficiencies may have a minor contribution to cause anemia in iron deficiency anemia patients. None of our 5 thalassemia trait patients had vitamin B12, iron or folic acid deficiency. The three microcytic anemia patients did not have iron deficiency, but one of them had vitamin B12 deficiency and another one had folic acid deficiency. Of 31 GPCA+/RAS patients, 3 (9.7%) had PA, 6 (19.4%) had vitamin B12 deficiency, and 3 (9.7%) had macrocytosis. Furthermore, 3 (50%) of 6 vitamin B12-deficient and 3 (100%) of 3 macrocytosis GPCA+/RAS patients had PA. Our findings indicate that only approximately 10—20% GPCA+/RAS patients had PA, vitamin B12 deficiency, or macrocytosis. Moreover, normocytic anemia (54.0%) and iron deficiency anemia (26.4%) are the two more common types of anemia in our RAS patients.

Of the 31 GPCA+/RAS patients, 3 (9.7%, group 1 patients) produced macrocytic RBCs, 25 (80.6%, group 2 patients) normocytic RBCs, and 3 (9.7%, group 3 patients) microcytic RBCs. Compared to group 2 patients, both groups 1 and 3 patients had relatively severe anemia, group 3 patients tended to have relatively low serum iron level, group 1 patients usually had low serum vitamin B12 level and significantly high blood homocysteine level, and group 3 patients usually had no vitamin B12 and folic acid deficiencies. A similar tendency was also observed in 240 Abs−/RAS patients including 6 macrocytosis (group 1), 200 normocytosis (group 2), and 34 microcytosis (group 3) Abs−/RAS patients.

GPCA+/RAS patients may tend to have intrinsic factor deficiency that finally results in vitamin B12 deficiency.[17—19] Therefore, it is interesting to know why 25 (80.6%) of our 31 GPCA+/RAS patients do not have vitamin B12 deficiency. It has been reported that supplementation with oral vitamin B12 is a safe and effective treatment for the correction of vitamin B12 deficiency state. Even when intrinsic factor is not present to aid the absorption of vitamin B12 or in other diseases that affect the usual absorption sites in the terminal ileum, oral supplement therapy with vitamin B12 remains effective.[8,18] Therefore, although patients have GPCA in their sera, enough dietary supply of vitamin B12 may prevent the occurrence of vitamin B12 deficiency in these 25 GPCA+/RAS patients. In addition, for those GPCA-positive patients it may need an enough long period of time to develop the autoimmune gastritis and finally resulting in complete failure of intrinsic factor production.[7,46] Therefore, for patients with lower serum titer of GPCA or those with medium or higher serum titer of GPCA of a shorter duration, their residual gastric parietal cells may still have some ability to produce intrinsic factors that help to absorb vitamin B12 from the small intestine.[12,17,18]

We conclude that the serum GPCA plays a significant role in causing vitamin B12 deficiency, higher MCV, and higher homocysteine level in GPCA+/RAS patients. Although the serum GPCA has some contributions to cause the anemia, it does not play a significant role in causing anemia in GPCA+/RAS patients. The disease of RAS itself does play a significant role in causing anemia and hematinic deficiencies in both GPCA+/RAS and Abs−/RAS patients.

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